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Shape-Selective Sensing of Lipids in Aqueous Solution by a Designed Fluorescent Molecular Tube

Berkeley J. Shorthill,† Christopher T. Avetta, and Timothy E. Glass*

Department of Chemistry, University of Missouri - Columbia, Columbia, Missouri 65211

Received April 23, 2004; E-mail: glasst@missouri.edu

Fluorescent probes have become a valuable means for investigating cellular events in real time via fluorescence microscopy. For such applications, chemical sensors have proven to be a valuable tool, particularly for tracking certain metal ions.[1] Chemical sensing of small organic compounds is less well developed, owing to the complexity of the cellular milieu. The major hurdle in this area is selectivity, that is preparing a sensor which responds only to the targeted analyte in the presence of many different potential competitors.[2]

Lipids are an important class of biomolecules for which artificial receptors have been prepared.[3] Indeed, cyclodextrins have been found to bind simple lipids in water via hydrophobic interactions.[4] Lipid sensing has many potential biochemical and biomedical applications, yet selectivity between various lipids is a daunting problem. Many different types of lipids vary only in the size and shape of their hydrophobic surface and lack distinct functional group handles. Herein we report a fluorescent tube-shaped sensor designed to selectively detect lipids on the basis of shape-selective[5] hydrophobic interactions.

We[6] and others[7] have been interested in extended calixarene motifs which use a naphthalene core. These calixnaphthalenes[8] appeared to be amenable to the construction of tube-like receptors which, when rendered water-soluble, would display an open hydrophobic cavity suitable for binding hydrophobic guests in a manner similar to that for the cyclodextrins. The distinct advantage of a calixarene-based receptor is the ability to control the cavity dimension and thus select for one type of lipid in preference to another on the basis of shape. Furthermore, the naphthalene core is fluorescent, endowing the receptor with a natural capacity for sensing.

Our initial sensor design (Figure 1) is based on a calixnaphthalene in which two bis-naphthalene clefts are linked together by amide connectors. The connectors were optimized[9] to produce an open cavity between the two clefts. There are two orientations for linking the two clefts: syn and anti. Modeling indicated that the syn isomer (**1a**) had a rigidly defined cavity owing to steric repulsion of the four OR groups on the lower portion of the receptor. The anti isomer (**1b**) was more open and flexible. Four acetic acid groups (R) were expected to impart water solubility. With four appended anionic groups, the syn isomer possesses a hydrophobic and a hydrophilic end, while the anti isomer is pseudosymmetrical at the ends.

The sensors were prepared as shown in Scheme 1. 2,6-Dihydroxynaphthalene was mono-alkylated with methyl bromoacetate. The resultant mono-phenol was allowed to react with the bis-acetal of malonaldehyde under acidic conditions to form the rigidly connected bis-naphthalene **2**.[10] Electrophilic formylation of the most activated peri-position on each naphthalene moiety of **2** produced compound **3**. This dialdehyde was oxidized to form the diacid (**4**). The dialdehyde could also be converted to diamine **5** by reductive amination. Amide coupling of **4** and **5** produced the diastereomeric sensors **1a** and **1b** following saponification of the methyl esters.

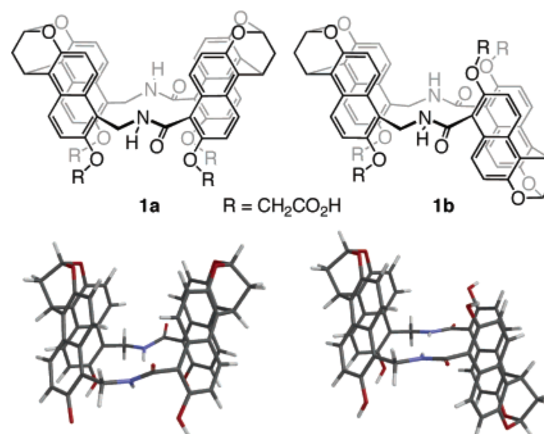
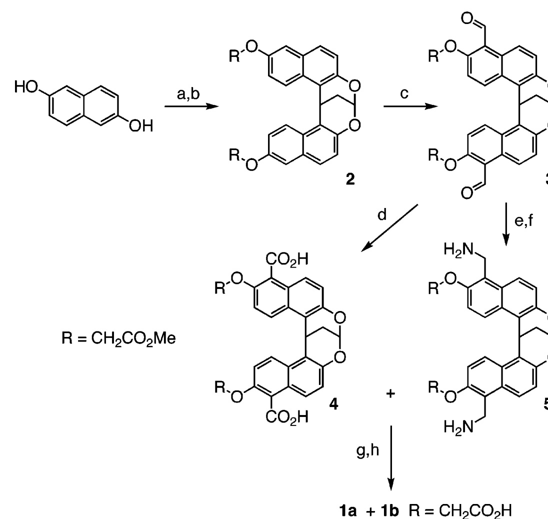


Figure 1. Three-dimensional representations of two molecular tubes. R groups are omitted from the models for clarity.

Scheme 1 [a]



[a] Reaction conditions: (a) NaH, methyl bromoacetate, DMF 33%; (b) malonaldehyde bis-dimethyl acetal, TFA, DCM, 76%; (c) α,α-dichloromethyl methyl ether, $TiCl_4$, DCM, 77%; (d) H_2NSO_3H, $NaClO_2$, Me_2CO/H_2O/THF, 95%; (e) *tert*-butyl carbamate, TFA, Et_3SiH, DCM, 66%; (f) TFA, quant. (g) PyBOP, $(i\text{Pr})_2NEt$, DMF, 35% (1.25:1 anti:syn); (h) NaOH, MeOH, 95%.

Initially, the binding properties of these molecular tubes were explored by NMR (40 mM Na_2CO_3 in D_2O). Fatty acids were used as guests to avoid attractive ionic interaction. Thus, the results tend to represent hydrophobic effects rather than electrostatic interactions. Both receptors **1a** and **1b** bound to various simple aliphatic acids as evidenced by shifts in the aromatic region of the NMR spectra. Interestingly, only in the case of the syn isomer did guest resonances appear at less than zero ppm, indicating that only in the syn isomer

† Current address: Alltech Associates, Inc., 2701 Carolean Industrial Dr., State College, PA 16801.

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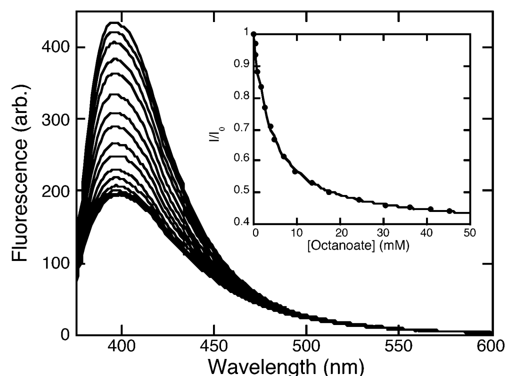

Figure 2. Fluorescence titration of compound **1a** with octanoic acid in buffer (20 mM HEPES; pH = 8.4; [**1a**] = 10^{-5} M; λ_{ex} = 365 nm). (Inset) Fit of the titration data at λ_{em} = 396 nm to a single-site binding isotherm.

Table 1. Association Constants and Fluorescence Changes of Sensors **1a** and **1b** with Lipid Guests[a]

		sensor **1a**		sensor **1b**	
entry	lipid	K_a (M^{-1})	I_{sat}/I_0[b]	K_a (M^{-1})	I_{sat}/I_0[b]
1	acetic acid	–	–	–	–
2	butyric acid	17	1.25	18	0.92
3	hexanoic acid	100	1.14	72	0.32
4	octanoic acid	250	0.39	190	0.25
5	decanoic acid	3,500	0.48	3,800	0.32
6	dodecanoic acid	18,000	0.47	27,000	0.31
7	*trans*-3-octenoic acid	92	0.61	110	0.26
8	4-methyl-octanoic acid	71	1.05	200	0.32
9	8-methyl-nonanoic acid	1,600	0.57	690	0.25
10	cyclohexane carboxylic acid	470	7.1	100	0.80
11	triethylene glycol	63	1.83	63	0.34
12	1-heptanol	4,900	0.28	6,700	0.19
13	*cis*-4-hepten-1-ol	1,200	1.32	5,200	0.58
14	heptylamine	16,000	0.42	16,000	0.11

[a] Measured by titration of **1** with the indicated lipid under the conditions listed in Figure 2. Error in K is ±10%. [b] I_{sat} is the fluorescence intensity at saturation taken from the fit of the titration data; I_0 is initial fluorescence intensity.

does the guest experience significant shielding by the naphthalene rings.

The recognition properties of the diastereomeric sensors were then probed spectroscopically in water at a pH of 8.4 to maintain solubility of all species. As described in prior work,[4a] experiments were carried out by titrating solutions of sensor (1−10 μM) with lipids such that the final concentration of each lipid never exceeded its critical micelle concentration.[11,12] Furthermore, over the range of concentrations used, both sensors exhibited Beer's law behavior, suggesting that the sensor itself did not aggregate under these conditions. Although no change in UV absorption was observed in either isomer upon addition of lipids, both isomers showed significant changes in fluorescence upon addition of fatty acids (Figure 2). Both the association constant and the maximum change in fluorescence were highly dependent on the guest and the isomer (Table 1). For the extended isomer **1b**, strong fluorescence quenching (42−89%) was observed for all hydrophobic guests except the two- and four-carbon acids (entries 1 and 2) and the cyclic acid (entry 10). The association constants varied uniformly as per the size and hydrophobicity of the guest with more hydrophobic guests binding more tightly. In the case of isomer **1a**, a pronounced difference in affinity pattern was observed. For simple straight-chain saturated lipids, the observed trend was in line with that observed for the anti isomer. Interestingly, introduction of branching in the middle of the chain inhibited binding (entry 8). However, branching at the end of a long chain was tolerated (entry 9). Alkenes in the chain give slightly lower binding constants with the *cis*-alkene (compare entries 12 and 13) being tolerated less well than the *trans*-alkene (entries 4 and 7). Finally, affinity increased from anionic to neutral to cationic guests as expected (entries 4, 12, and 14).

The unusual feature of the fluorescence data for the syn isomer (**1a**) is the type of fluorescence change observed, wherein some guests induced a fluorescence *quenching* and some induced a fluorescence *enhancement*. Examination of Table 1 reveals that in the case of **1a** only straight-chain lipids which can thread completely through the tube produced a quenched fluorescence (39−72%) similar to that seen in **1b**. Guests with branching, rings, or *cis*-alkenes (indeed the straight-chain hydrophilic triethylene glycol) produced mild to marked fluorescent enhancements (5−710%). Even short-chain lipids (entries 2 and 3) which cannot span the length of the sensor gave a fluorescence increase. Thus, sensor **1a** possesses an unusual selectivity for long, straight-chain hydrophobic lipids.

The pair of diastereomeric molecular tubes **1a** and **1b** represent competent sensors for all types of lipids since they recognize only the hydrophobic surface and no other functional group. In particular, the syn isomer **1a** reports on the presence of hydrophobic compounds with shape-selective recognition by fluorescent quenching for straight-chain lipids and fluorescent enhancement for other lipids. To our knowledge, this type of spectroscopic selection between simple lipids based only on the shape of their hydrophobic surface is unprecedented. Although the NMR spectra of the complexes between receptor **1a** and straight-chain and branched lipids indicate differences in bound-state conformation, the origin of the differences in fluorescent responses remains unclear. The mechanism of this fluorescence modulation is currently under investigation.

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Supporting Information Available: Experimental details and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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